547. *Steroids. Part I.* 11-*Oxygenated Steroids from Ergosteryl-D Acetate.*

By Richard Budziarek, G. T. Newbold, Robert Stevenson, and F. S. Spring.

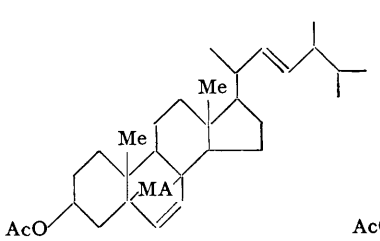
Experiments are described in which 11-hydroxy-steroids and an 11-keto-steroid are obtained from ergosterol. Whereas oxidation of ergosteryl-D acetate with one mol. of performic acid gives 3β-acetoxyergosta-9(11) : 22-dien-7-one (XI), with two mols. of the oxidising agent 3β-acetoxy-9α : 11α-epoxyergost-22-en-7-one (XIV) is obtained. By using mild alkaline conditions, hydrolysis of the latter is accompanied by rearrangement to give 3β : 11α-dihydroxyergosta-8 : 22-dien-7-one (XV; R′ = H). Treatment of 3β-acetoxy-9α : 11α-epoxyergost-22-en-7-one with strong alkali also effects a rearrangement, to give in this case, after acetylation, 7 : 11-diketoergost-22-en-3β-yl acetate (XIII). 7 : 11-Diketoergost-22-en-3β-yl acetate is also obtained by treatment of 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one (XV; R′ = Ac) with strong alkali followed by acetylation of the product. Ergosteryl-D acetate epoxide has been converted into 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one (XVI; R′ = H) characterised by its acetyl derivative (XVI; R′ = Ac) and by its ready conversion into 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one (XV; R′ = Ac).

The experiments described in this paper and in some succeeding Parts of this series have as their object the development of routes to cortisone starting from ergosterol. The investigation was commenced in July, 1949, at a time when the only practicable preparative method for cortisone started from deoxycholic acid and proceeded by a tortuous route involving more than thirty stages.

Bergmann and Stevens (*J. Org. Chem.*, 1948, **13**, 10; cf. Bergmann and Klacsmann, *ibid.*, p. 21) suggested the use of ergosterol as a starting material for the partial synthesis of adrenal cortical steroids "because of the comparative ease with which it may be converted to derivatives like dehydroergosterol which possess unsaturation at C_{11} and which might lend themselves to the introduction of oxygen at this point. In addition the 22 : 23-double bond was expected to facilitate removal of the side-chain to permit its replacement by one of the typical side-chains of adrenal cortical hormones." In an elegant series of experiments, Bergmann and Stevens made considerable progress in the last direction. They showed that protection of the conjugated nuclear double bonds of ergosteryl acetate by means of maleic anhydride allowed the 22 : 23-double bond to be preferentially oxidised. Thus treatment of the maleic anhydride adduct (I) with ozone gave an aldehyde (II) which was converted into the enol acetate (III), ozonolysis of which, followed by pyrolysis of the product, gave 3β-acetoxypregna-5 : 7-dien-20-one (IV) (cf. Antonucci, Bernstein, Giancola, and Sax, *J. Org. Chem.*, 1951, **16**, 1356). Less successful were attempts to introduce either a hydroxyl or a ketone group at the 11-position starting from dehydroergosteryl acetate–maleic anhydride 22 : 23-dibromide. Although the epoxide (V) was obtained, pyrolysis of this was accompanied by aromatisation of ring B.

Our approach to the preparation of an 11-oxygenated steroid started from ergosteryl-D acetate [ergosta-7 : 9(11) : 22-trien-3β-yl acetate] (VI), the simplest available derivative of ergosteryl acetate containing unsaturation involving $C_{(11)}$ and a side-chain ethylenic linkage. After a considerable portion of the work described in Parts I and II of this series had been completed, a number of preliminary announcements were made describing the conversion of 7 : 9(11)-dienic steroids into 11-oxygenated steroids (Chamberlin, Ruyle, Erickson, Chemerda, Aliminosa, Erickson, Sita, and Tishler, *J. Amer. Chem. Soc.*, 1951, **73**, 2396; Fieser, Herz, and Huang, *ibid.*, p. 2397; Stork, Romo, Rosenkranz, and Djerassi, *ibid.*, p. 3546; Djerassi, Mancera, Stork, and Rosenkranz, *ibid.*, p. 4496; Fieser, Babcock, Herz, Huang, and Schneider, *ibid.*, p. 4053) and more recently a detailed paper on the same subject has appeared (Heusser, Eichenberger, Kurath, Dällenbach, and Jeger, *Helv. Chim. Acta*, 1951, **34**, 2106). A preliminary note on some of the results described in this and the following paper has been published (*Chem. and Ind.*, 1951, 1035).

The oxidation of ergosteryl-D acetate (VI), obtainable from 5-dihydroergosteryl acetate (VII) by methods described in Part II, with chromium trioxide in acetic acid has



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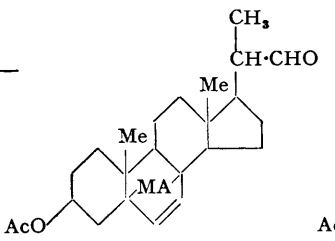

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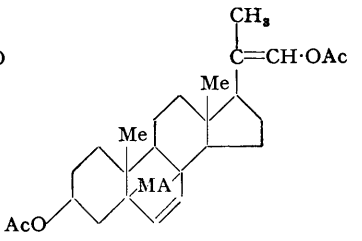

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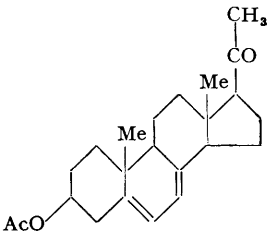

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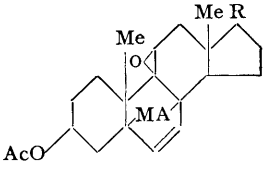

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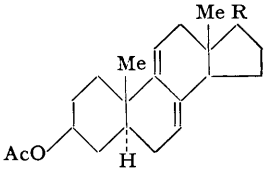
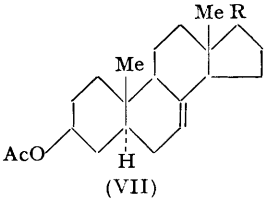


(VI)



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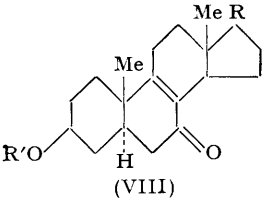
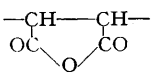


(VIII)

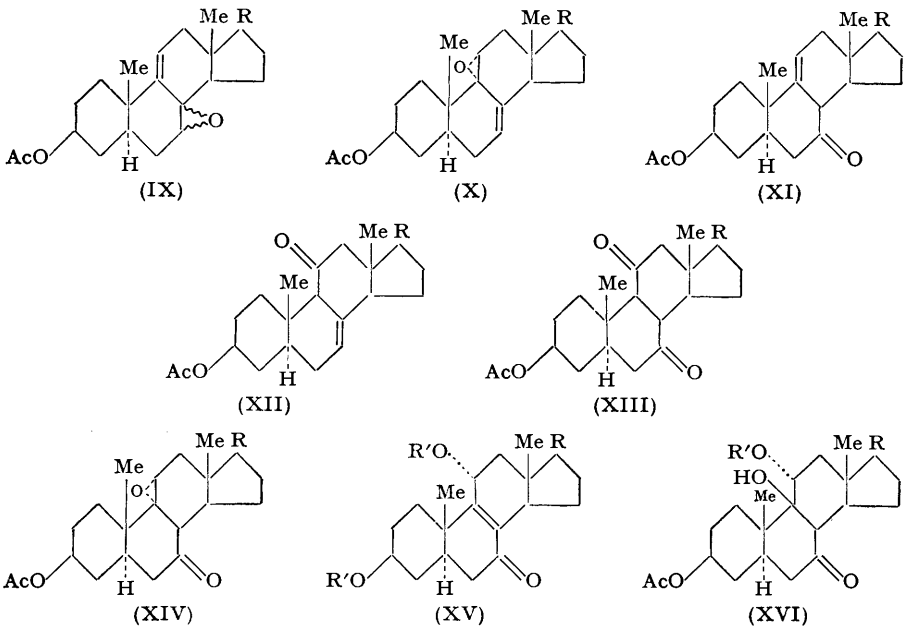
—MA— = 

R = —CHMe·CH:CH·CHMe·CHMe$_2$

been examined under a variety of conditions. In all cases, 3β-acetoxyergosta-8 : 22-dien-7-one (VIII; R′ = Ac) was obtained in poor yield. This αβ-unsaturated ketone was first obtained as a minor product of the oxidation of 5-dihydroergosteryl acetate (VII) with chromium trioxide (Stavely and Bollenback, *J. Amer. Chem. Soc.*, 1943, **65**, 1290). In addition to 3β-acetoxyergosta-8 : 22-dien-7-one, oxidation of ergosteryl-D acetate with chromic anhydride gives, in very small yield, a compound $C_{30}H_{44}O_4$, m. p. 127—128°, $[\alpha]_D$ −32°, which was not obtained in sufficient amount to allow a detailed investigation.

In view of the unpromising yields of the oxidation products described above, the oxidation of ergosteryl-D acetate with hydrogen peroxide in formic acid was investigated. With one mol. of performic acid, a compound, $C_{30}H_{46}O_3$, was obtained in good yield. This compound gives a pale yellow colour with tetranitromethane from which it follows that it is formed by addition of oxygen to the conjugated system of ergosteryl-D acetate since the last compound, in common with many conjugated dienes, gives a dark-brown colour with tetranitromethane. In support of this decision it was found that the compound $C_{30}H_{46}O_3$ does not exhibit absorption of high intensity above 2200 Å. The initial reaction product is unstable, simple crystallisation being accompanied by the appearance of selective light absorption with a maximum at 2540 Å. Hydrolysis of the initial reaction product with either dilute alkali or mineral acid is accompanied by rearrangement to give 3β-hydroxyergosta-8 : 22-dien-7-one (VIII; R′ = H). If the formation of a 7 : 11-oxide be excluded as sterically improbable, oxidation has occurred at either the 9 : 11- or at the 7 : 8-ethylenic bonds of ergosteryl-D acetate to give either an epoxide [(IX) or (X)] or a ketone [(XI) or

(XII)]. The 9α : 11α-configuration is ascribed to (X) because of the well-established preferential rear attack by reagents at the 9- and the 11-position (see Fieser, *Experientia*, 1950, **6**, 312). The ease with which the compound, $C_{30}H_{46}O_3$, is rearranged to an αβ-unsaturated ketone by alkali (and also by simple crystallisation) is in marked contrast to the stability of the known ergosteryl-D acetate epoxide. The last compound is obtained by the action of one mol. of perbenzoic acid on ergosteryl-D acetate (Chamberlin *et al.*, *loc. cit.*) and it has been ascribed the structure 9α : 11α-epoxyergosta-7 : 22-dien-3β-yl acetate (X) by Heusser *et al.* (*loc. cit.*) who prepared it by the action of monoperphthalic acid on ergosteryl-D acetate. In the preliminary communication from this laboratory (*loc. cit.*) the oxide was given the alternative structure 7ξ : 8ξ-epoxyergosta-9(11) : 22-dien-3β-yl acetate (IX). Until a satisfactory proof of structure is available, we will describe this compound as ergosteryl-D acetate epoxide. We believe that the difference in reactivity between ergosteryl-D acetate epoxide and the compound $C_{30}H_{46}O_3$ shows that the latter is not an epoxide [(IX) or (X)], but rather a ketone [(XI) or (XII)]. That hydrolysis and rearrangement with alkali give 7-ketoergosta-8 : 22-dien-3β-ol excludes (XII), and it is concluded that the compound $C_{30}H_{46}O_3$ is 7-ketoergosta-9(11) : 22-dien-3β-yl acetate (XI). These views are supported by the observation of Djerassi *et al.* (*loc. cit.*) that treatment of the performic acid mother-liquors, obtained from the conversion of 20-keto*allo*pregna-7 : 9(11)-dien-3β-yl acetate into 9α : 11α-epoxy-7 : 20-diketo*allo*pregnan-3β-yl acetate, with alkali gave 7 : 20-diketo*allo*pregn-8-en-3β-ol presumably by rearrangement of the $\Delta^{9(11)}$-7 : 8-oxide and/or the $\Delta^{9(11)}$-7-ketone. Again, Fieser, Babcock, Herz, Huang, and Schneider (*loc. cit.*; cf. Fieser, Herz, and Huang, *loc. cit.*) have shown that oxidation of methyl 3α-acetoxychola-7 : 9(11)-dienoate with sodium dichromate dihydrate gives methyl 3α-acetoxy-7-ketochol-9(11)-enate which, like the oxidation product from ergosteryl-D acetate, is readily isomerised by alkali to the corresponding Δ^8-7-ketone.



(R = ·CHMe·CH:CH·CHMe·CHMe$_2$)

Oxidation of ergosteryl-D acetate with two mols. of performic acid gave a compound, $C_{30}H_{46}O_4$, which does not show selective absorption of high intensity above 2200 Å. Since the primary product of the performic acid oxidation of ergosteryl-D acetate has been shown to be (XI) the compound $C_{30}H_{46}O_4$ is either 7 : 11-diketoergost-22-en-3β-yl acetate (XIII) or 9α : 11α-epoxy-7-ketoergost-22-en-3β-yl acetate (XIV). The oxidation product is

however different from the 7 : 11-diketone (XIII) (Chamberlin *et al.* and Heusser *et al.*, *locc. cit.*). It is therefore 9α : 11α-epoxy-7-ketoergost -22-en-3β-yl acetate (XIV). This compound can also be prepared from 7-ketoergosta-9(11) : 22-dien-3β-yl acetate (XI) by protection of the 22 : 23-ethylenic linkage by the addition of one mol. of bromine, followed by oxidation with perbenzoic acid and debromination of the product with zinc. Relatively mild alkaline hydrolysis of 9α : 11α-epoxy-7-ketoergost-22-en-3β-yl acetate gives a compound $C_{28}H_{44}O_3$ shown to be 3β : 11α-dihydroxyergosta-8 : 22-dien-7-one (XV; R′ = H) by the reactions now to be discussed. The structure ascribed to the compound $C_{28}H_{44}O_3$ is supported by its formation from (XIV), by its ready conversion into a diacetate and by the ultra-violet absorption spectrum [maxima at 2520 Å (ε = 9000)]. Treatment of the diacetate of the compound $C_{28}H_{44}O_3$ with strong aqueous-ethanolic potassium hydroxide followed by acetylation yielded a reaction mixture readily separable into two components by chromatography on alumina. One of these proved to be identical with 7 : 11-diketoergost-22-en-3β-yl acetate (XIII), the identity being established by direct comparison with a specimen prepared as described by Heusser *et al.* (*loc. cit.*). A similar isomerisation of 6-ketocholest-4-en-3β-yl acetate into cholestane-3 : 6-dione was observed by Heilbron, Jones, and Spring (*J.*, 1937, 801) and the conversion of 6β : 21-diacetoxypregn-4-ene-3 : 20-dione into 21-hydroxy*allo*pregnane-3 : 6 : 20-trione by treatment with alkali has been reported by Herzig and Ehrenstein (*J. Org. Chem.*, 1951, **16**, 1050). The isolation of 7 : 11-diketoergost-22-en-3β-yl acetate shows that the compound $C_{28}H_{44}O_3$ is either 3β : 11-dihydroxyergosta-8 : 22-dien-7-one (XV; R′ = H) or 3β : 7-dihydroxyergosta-8 : 22-dien-11-one. If our identification of the performic oxidation product $C_{30}H_{46}O_4$ as 9α : 11α-epoxy-7-ketoergost-22-en-3β-yl acetate is correct, the latter possibility is excluded, and the compound $C_{28}H_{46}O_4$ must be 3β : 11-dihydroxyergosta-8 : 22-dien-7-one (XV; R = H). Concerning the orientation of the 11-hydroxyl group in 3β : 11-dihydroxyergosta-8 : 22-dien-7-one the ease of acetylation to a diacetate at first sight precludes the 11β-configuration although this deduction may be invalidated by the effect of the 8 : 9-ethylenic linkage upon the accessibility of an 11β-hydroxyl group. An argument based on analogy can be adduced in favour of the 11α-configuration. Oxidation of 3β : 20β-diacetoxy-*allo*pregna-7 : 9(11)-diene with performic acid gives 3β : 20β-diacetoxy-9α : 11α-epoxy-*allo*pregnan-7-one, alkaline hydrolysis of which yields 3β : 11α : 20β-trihydroxypregn-8-en-7-one which gives a triacetate. Catalytic hydrogenation of 3β : 11α : 20β-trihydroxypregn-8-en-7-one gives 3β : 11α : 20β-trihydroxypregnan-7-one which forms a triacetate (Stork *et al.*, *loc. cit.*; cf. Djerassi *et al.*, *loc. cit.*). Whilst it can be reasoned that the conversion of a 3 : 11 : 20-trihydroxypregn-8-en-7-one into a triacetate does not prove that the 11-hydroxyl group has the α-configuration, since a neighbouring-group effect upon an 11β-hydroxyl group may be shown by an 8 : 9-unsaturated centre (cf. Heymann and Fieser, *J. Amer. Chem. Soc.*, 1951, **73**, 5252), triacetylation of the saturated 3 : 11 : 20-trihydroxypregnan-7-one proves the α-configuration for the 11-hydroxyl group in these compounds. Consequently, the 11-hydroxyl group in 3β : 11-dihydroxyergosta-8 : 22-dien-7-one, obtained in an analogous manner from ergosteryl-D acetate, is assigned the α-configuration.

7 : 11-Diketoergost-22-en-3β-yl acetate is accompanied by a second reaction product, $C_{32}H_{52}O_5$. This compound gives a faint yellow colour with tetranitromethane, does not show high-intensity selective absorption above 2200 Å., and contains an ethoxyl group; a discussion of the nature of this compound is deferred until further data are available. Treatment of 3β-acetoxy-9α : 11α-epoxyergost-22-en-7-one with concentrated aqueous-ethanolic potassium hydroxide solution followed by acetylation of the reaction product also gives a mixture of 7 : 11-diketoergost-22-en-3β-yl acetate (XIII) and the ethoxy-compound $C_{32}H_{52}O_5$.

Parallel with the chromium trioxide and performic acid oxidation experiments described above a study was made of the oxidation of ergosteryl-D acetate with perbenzoic acid. We have been anticipated in a description of a monoxide of ergosteryl-D acetate which is obtained by the action of one mol. of perbenzoic acid (Chamberlin *et al.*, and Heusser *et al.*, *locc. cit.*). Ergosteryl-D acetate epoxide is smoothly hydrolysed with alkali to the corresponding ergosterol-D epoxide, which was also obtained in an attempt to reduce ergosteryl-D

acetate epoxide with lithium aluminium hydride. With two mols. of perbenzoic acid we have not succeeded in isolating a homogeneous reaction product, apart from a small amount of ergosteryl-D acetate monoxide. In an attempt to correlate ergosteryl-D acetate monoxide with 3β-acetoxy-9α : 11α-epoxyergost-22-en-7-one, a solution of the former was treated with one mol. of bromine and then with an excess of perbenzoic acid. Debromination of the reaction mixture gave 3β-acetoxyergosta-8 : 22-dien-7-one and, in low yield, a crystalline compound $C_{30}H_{48}O_5$, identified as 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one (XVI; R = H) by the reactions described below. The compound does not exhibit selective high-intensity ultra-violet absorption above 2200 Å. It is acetylated under normal conditions to 3β : 11α-diacetoxy-9β-hydroxyergost-22-en-7-one (XVI; R = Ac). Treatment of this acetyl derivative with dilute potassium hydroxide solution followed by acetylation gives 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one (XV; R = Ac) identical with the compound obtained as described above. The two hydroxyl groups in 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one are considered to be *trans*-orientated with respect to each other, since they almost certainly originate by a hydrolytic cleavage of a 9α : 11α-oxide intermediate. The infra-red absorption spectrum of 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one, for which we are indebted to Dr. I. A. Brownlie, shows bands at 1732 (3β-acetate group), 1710 (7-ketone group) and at 3400 cm.$^{-1}$ (hydroxyl groups).

Experimental

Specific rotations were determined in chloroform solution in a 1-dm. tube at approx. 15°. Ultra-violet absorption spectra were measured in ethanol solution with a Unicam SP. 500 spectrophotometer.

3β-*Acetoxyergosta*-8 : 22-*dien*-7-*one* (VIII; R′ = Ac).—(*a*) Ergosteryl-D acetate (2·19 g.) in stabilised glacial acetic acid (200 c.c.) was treated dropwise, during 45 minutes at 95° with stirring, with a solution of chromium trioxide in 95% acetic acid (20 c.c.; 1·044N). After a further hour's stirring the reaction mixture was evaporated to small bulk under reduced pressure, diluted with water, and extracted with ether. The ethereal extract was washed successively with water, potassium hydroxide solution (5%), and water, and dried (Na_2SO_4). Removal of the ether gave a yellow semicrystalline solid which on trituration with warm methanol (100 c.c.) yielded a pale yellow solid (800 mg.), m. p. 173—176°, which gave a brown colour with tetranitromethane in chloroform. One crystallisation from chloroform–methanol gave ergosteryl-D acetate as blades, m. p. 175—176°, alone or mixed with an authentic specimen. From the methanolic mother-liquor a yellow solid (310 mg.), m. p. 152—162°, was obtained which gave a yellow colour with tetranitromethane in chloroform. Recrystallisation of this solid from methanol (10 c.c.) yielded plates (110 mg.), m. p. 168—170°, a solution of which in light petroleum (b. p. 60—80°) was chromatographed on a column (12 × 1·5 cm.) of alumina (Grade II). After washing of the column with light petroleum (b. p. 40—60°) and light petroleum (b. p. 40—60°)–benzene (4 : 1), elution with light petroleum (b. p. 40—60°)–benzene (2 : 1; 50 c.c.) gave ergosteryl-D acetate (15 mg.), m. p. 165—170°. Elution with light petroleum (b. p. 40—60°)–benzene (2 : 1; 50 c.c.) and then with light petroleum–benzene (1 : 1; 50 c.c.) gave a solid (50 mg.) from which, after one crystallisation from methanol, 3β-acetoxyergosta-8 : 22-dien-7-one was obtained as plates, which after sublimation at 160—170°/5 × 10^{-4} mm. had m. p. 203°, undepressed when mixed with the specimen described below, and $[\alpha]_D$ −51° (*c*, 1·2) (Found: C, 79·4; H, 10·5. Calc. for $C_{30}H_{46}O_3$: C, 79·2; H, 10·2%). Light absorption: Max. at 2520 Å (ε = 10,000).

Alkaline hydrolysis of the acetate gave 3β-*hydroxyergosta*-8 : 22-*dien*-7-*one* which separated from methanol as plates, m. p. 175—177° undepressed by the specimen described below, $[\alpha]_D$ −42° (*c*, 0·8) (Found: C, 78·0; H, 11·1. $C_{28}H_{44}O_2,CH_3{\cdot}OH$ requires C, 78·3; H, 10·9%). Light absorption: Max. at 2520 Å (ε = 11,100).

A further quantity (42 mg.) of 3β-acetoxyergosta-8 : 22-dien-7-one was obtained by elution of the column with light petroleum (b. p. 40—60°)–benzene (1 : 1; 220 c.c.).

(*b*) A solution of ergosteryl-D acetate (2·19 g.) in warm stabilised acetic acid (220 c.c.) was rapidly chilled with stirring. A solution of chromium trioxide in acetic acid (20 c.c.; 1·044N) was added dropwise during 4 hours at 15° to the stirred suspension. Stirring was continued for 1½ hours whereafter solution was complete. After the addition of more chromium trioxide in acetic acid (10 c.c.; 1·044N) during 20 minutes, the solution was kept at room temperature for

60 hours. Methanol (2 c.c.) was added and the acetic acid removed under reduced pressure. The neutral fraction isolated in the usual manner formed a light brown resin which solidified on trituration with methanol. The solid (610 mg.), m. p. 154—166°, gave a pale yellow colour with tetranitromethane in chloroform and showed light absorption maxima at 2640 and 2520 Å. The solid (600 mg.) in light petroleum (b. p. 40—60°)–benzene (10 c.c.; 4 : 1) was chromatographed on a column of alumina (Grade II; 10 × 2 cm.). Washing with the same solvent mixture (500 c.c.) was followed by elution with light petroleum (b. p. 40—60°)–benzene (500 c.c.; 2 : 1), to give a yellow solid (205 mg.). Slow evaporation of a methanol solution of this solid gave a mixture of long yellow needles and short colourless needles which were separated mechanically. Sublimation of the yellow needles (8·2 mg.) in a high vacuum gave a *compound* (5·1 mg.), m. p. 125—127°, $[\alpha]_D$ −32° (*c*, 0·9) (Found: C, 76·3; H, 10·1. $C_{30}H_{44}O_4$ requires C, 76·9; H, 9·5%). Light absorption: Max. at 2650 Å (ε = 4700). Elution of the column with light petroleum (b. p. 40—60°)–benzene (700 c.c.; 2 : 1) yielded a solid (360 mg.), m. p. 190—192°, which on crystallisation from methanol gave 3β-acetoxyergosta-8 : 22-dien-7-one.

(*c*) A solution of ergosteryl-D acetate (2·19 g.) in benzene (20 c.c.) and stabilised acetic acid (150 c.c.) was kept at 50° and treated with a solution of chromium trioxide in glacial acetic acid (31 c.c.; 1·044 N) added during 1 hour with stirring. After 1 hour's stirring at 50° the mixture was concentrated under reduced pressure to 20 c.c. and diluted with water. The precipitated solid was isolated by means of ether. The neutral fraction (0·63 g.) was twice crystallised from methanol and then chromatographed in light petroleum (b. p. 40—60°)–benzene (5 : 1) on a column of activated alumina (Grade II), to give 3β-acetoxyergosta-8 : 22-dien-7-one which after crystallisation from methanol separated as plates, m. p. 208—211°, $[\alpha]_D$ −56° (*c*, 0·5) (Found: C, 79·4; H, 10·5. Calc. for $C_{30}H_{46}O_3$: C, 79·2; H, 10·2%); it gives a light yellow colour with tetranitromethane in chloroform. Light absorption: Max. at 2540 Å (ε = 10,100).

A second crop (0·36 g.) from the methanol mother-liquors was chromatographed on alumina. A fraction, eluted by light petroleum (b. p. 40—60°)–benzene (3 : 1), was crystallised from methanol, to give the compound $C_{30}H_{44}O_4$ (50 mg.), as needles, $[\alpha]_D$ −30° (*c*, 0·9), m. p. 127—128·5° undepressed on admixture with the specimen described above. Elution of the chromatogram with light petroleum (b. p. 40—60°)–benzene (1 : 1) gave 3β-acetoxyergosta-8 : 22-dien-7-one (180 mg.).

(*d*) Ergosteryl-D acetate (2·19 g.) suspended in acetic acid (200 c.c.) was stirred at 15° and treated dropwise during 1 hour with a solution of chromium trioxide in acetic acid (30 c.c.; 1·044N) containing sulphuric acid (2·5 c.c.; *d* 1·84). After a second hour's stirring the reaction mixture was treated as described under (*c*). Four crystallisations of the neutral fraction (0·7 g.) from methanol followed by chromatography on alumina gave 3β-acetoxyergosta-8 : 22-dien-7-one as plates, m. p. 208—210° (from methanol), $[\alpha]_D$ −53° (*c*, 1·1). Light absorption: Max. at 2540 Å (ε = 10,100). The methanolic mother-liquors were combined and evaporated and the residue (1 g.) was chromatographed on alumina, to give the compound $C_{30}H_{44}O_4$ (35 mg.), $[\alpha]_D$ −29° (*c*, 0·4). Light absorption: Max. at 2650 Å (ε = 4300). The m. p. (123—125°) was undepressed when the substance was mixed with the specimen described above. Later fractions from the chromatogram gave 3β-acetoxyergosta-8 : 22-dien-7-one (130 mg.).

3β-*Acetoxyergosta*-9(11) : 22-*dien*-7-*one* (XI).—A mixture of ergosteryl-D acetate (2·2 g.) in benzene (20 c.c.), formic acid (20 c.c.; 90%), and hydrogen peroxide (0·65 c.c.; 30%) was stirred for 20 hours at 15°. The reaction mixture was evaporated under reduced pressure below 50° (bath-temp.) and the residue crystallised from methanol, to give 3β-*acetoxyergosta*-9(11) : 22-*dien*-7-*one* (930 mg.) as needles, m. p. 194—197°, $[\alpha]_D$ +20° (*c*, 0·5) (Found: C, 78·8; H, 10·2. $C_{30}H_{46}O_3$ requires C, 79·2; H, 10·2%); it gives a pale yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å. Repeated crystallisation from methanol did not appreciably alter the m. p. but caused the appearance of high-intensity absorption at 2540 Å.

3β-*Hydroxyergosta*-8 : 22-*dien*-7-*one* (VIII; R′ = H).—(*a*) 3β-Acetoxyergosta-9(11) : 22-dien-7-one (250 mg.) was heated under reflux with aqueous methanolic potassium hydroxide (15 c.c.; 3%) for 2 hours. Isolation of the product by means of ether, followed by crystallisation from methanol. gave 3β-hydroxyergosta-8 : 22-dien-7-one (150 mg.) as plates, m. p. 176—178° (alone or mixed with the specimen described above), $[\alpha]_D$ −43° (*c* 1·3). Light absorption: Max. at 2540 Å (ε = 10,000) (Found: C, 77·9; H, 10·8. Calc. for $C_{28}H_{44}O_2,CH_3{\cdot}OH$: C, 78·3; H, 10·9%).

(*b*) 3β-Acetoxyergosta-9(11) : 22-dien-7-one was heated under reflux for 2 hours with 1% aqueous methanolic hydrogen chloride. The product isolated in the usual manner gave 3β-hydroxyergosta-8 : 22-dien-7-one as plates (from methanol), m. p. 180—182°, $[\alpha]_D$ −44° (*c*, 0·7) (Found: C, 77·9; H, 10·8%).

3β-*Acetoxy*-9α : 11α-*epoxyergost*-22-*en*-7-*one* (XIV).—(*a*) Ergosteryl-D acetate (2·2 g.) in benzene (20 c.c.) was stirred with a mixture of formic acid (20 c.c.; 90%) and hydrogen peroxide (1·2 c.c.; 30%) for 20 hours at 15°. The reaction mixture was evaporated under reduced pressure below 50°. Crystallisation of the residue from methanol gave 3β-*acetoxy*-9α : 11α-*epoxyergost*-22-*en*-7-*one* (360 mg.) as needles (which formed slowly from an initial gel), m. p. 220—223°, $[\alpha]_D$ −85° (*c*, 0·5) (Found: C, 76·2; H, 9·8. $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%). The compound gives a pale yellow colour with tetranitromethane in chloroform and does not exhibit high-intensity absorption above 2200 Å.

(*b*) 3β-Acetoxyergosta-9(11) : 22-dien-7-one (500 mg.) in dry chloroform (25 c.c.) was treated during 1½ hours with stirring at 0° with a solution of bromine (183 mg.) in chloroform (4 c.c.). A solution of perbenzoic acid (1·5 mol.) in chloroform (4·5 c.c.) was added and the whole kept at 0° for 3 days. The chloroform was removed under reduced pressure below 35° and the residue debrominated by heating it with zinc dust (7 g.) in acetic acid (30 c.c.) on the steam-bath for 6 hours. A solution of the product, isolated by means of ether, in benzene (ca. 20 c.c.) was chromatographed on a column of Grade II alumina (7·5 × 1 cm.), which was washed with benzene (75 c.c.). Removal of the solvent gave 3β-acetoxy-9α :11α-epoxyergost-22-en-7-one (130 mg.), separating from methanol as needles, m. p. 222—223° (alone or mixed with the specimen described above), $[\alpha]_D$ −79° (*c*, 0·8) (Found: C, 76·55; H, 9·9%). The compound showed the characteristic gelation described above.

3β : 11α-*Diacetoxyergosta*-8 : 22-*dien*-7-*one* (XV; R′ = Ac).—3β-Acetoxy-9α : 11α-epoxyergost-22-en-7-one (95 mg.) was heated under reflux for 1 hour in aqueous methanolic potassium hydroxide (6 c.c.; 3%). The product was isolated by means of ether and crystallised from acetone, to give 3β : 11α-*dihydroxyergosta*-8 : 22-*dien*-7-*one* (60 mg.) as needles, m. p. 215°, $[\alpha]_D$ −6° (*c*, 1·5, 1·1) (Found: C, 78·45; H, 10·35. $C_{28}H_{44}O_3$ requires C, 78·75; H, 10·7%). The compound gives a faint yellow colour with tetranitromethane in chloroform. Light absorption: Max. at 2540 Å (ε = 8100).

This product (50 mg.) was heated on the steam-bath with acetic anhydride (1 c.c.) and pyridine (1 c.c.) for 1 hour. Isolation by means of ether gave 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one (XV; R = Ac) (35 mg.) which separated from methanol as flat needles, m. p. 175—177°, $[\alpha]_D$ +13° (*c*, 0·5) (Found: C, 74·8; H, 9·7. $C_{32}H_{48}O_5$ requires C, 75·0; H, 9·4%). It gives a light yellow colour with chloroformic tetranitromethane and exhibits a light absorption maximum at 2520 Å (ε = 10,400).

Ergosteryl-D Acetate Epoxide.—Ergosteryl-D acetate (9·0 g.) in chloroform (35 c.c.) was treated with perbenzoic acid (1·36 mols.) in chloroform (110 c.c.) added with stirring during 3—4 hours. The mixture was kept at 0° for 12 hours. The solid residue, obtained by removal of the solvent at room temperature under reduced pressure, was dissolved in the minimum volume of boiling acetone. On cooling, the solution deposited ergosteryl-D acetate epoxide as hexagonal plates (3·9 g.), m. p. 205—207°, which after two recrystallisations from the same solvent had m. p. 211—213°, $[\alpha]_D$ −38° (*c*, 2·2) (Found: C, 79·5; H, 10·3. Calc. for $C_{30}H_{46}O_3$: C, 79·2; H, 10·2%). It gives a yellow colour with tetranitromethane in chloroform and does not show selective absorption of high intensity above 2200 Å. Chamberlin *et al.* (*loc. cit.*) report m. p. 202—205°, $[\alpha]_D$ −35°, Heusser *et al.* (*loc. cit.*) report m. p. 205—207°, $[\alpha]_D$ −39·5°.

Ergosterol-D Epoxide.—(*a*) Ergosteryl-D acetate epoxide (400 mg.) was heated under reflux with aqueous methanolic potassium hydroxide (50 c.c.; 2%) and methanol (10 c.c.) for 2 hours. The solid (330 mg.) which separated on cooling was washed with water and twice crystallised from methanol, to give *ergosterol-D epoxide* as flat needles, m. p. 187—189°; $[\alpha]_D$ −41° (*c*, 1·3) (Found: C, 78·5; H, 10·8. $C_{28}H_{44}O_2,CH_3{\cdot}OH$ requires C, 78·3; H, 10·9%).

Acetylation of ergosterol-D epoxide (105 mg.) was effected by heating on the steam-bath for 1 hour with acetic anhydride (2 c.c.) and pyridine (1 c.c.). The solid which separated on dilution with water was twice crystallised from acetone, to give ergosteryl-D acetate epoxide as laminæ, m. p. 210—212° alone or mixed with an authentic specimen; $[\alpha]_D$ −35° (*c*, 1·0) (Found: C, 79·3; H, 10·3. Calc. for $C_{30}H_{46}O_3$: C, 79·2; H, 10·2%).

(*b*) Ergosteryl-D acetate epoxide (500 mg.) in absolute tetrahydrofuran (15 c.c.) was added dropwise during 15 minutes to a vigorously stirred, refluxing solution of lithium aluminium hydride (1·0 g.) in tetrahydrofuran (100 c.c.). The mixture was refluxed for 3 hours, stored overnight at room temperature, diluted with ether, and neutralised by the addition of 10% sulphuric acid. The ethereal phase was washed successively with water, sodium hydrogen carbonate solution, and water, and dried (Na_2SO_4). Removal of the ether gave a solid which after two recrystallisations from methanol gave ergosterol-D epoxide, m. p. 182—185° alone or mixed with the specimen described above.

3β-*Hydroxyergosta*-8 : 22-*dien*-7-*one* (VIII; R′ = H).—Ergosteryl-D acetate epoxide (150 mg.) was refluxed in aqueous methanolic hydrogen chloride (10 c.c.; 0·7%) for 2 hours. The solution was concentrated and the solid (85 mg.) which separated on cooling crystallised thrice from methanol, to give 3β-hydroxyergosta-8 : 22-dien-7-one as plates, m. p. 173—175° (undepressed with the specimen described above), $[\alpha]_D$ −45° (*c*, 0·5) (Found: C, 78·5; H, 11·1. Calc. for $C_{28}H_{44}O_2,CH_3{\cdot}OH$: C, 78·3; H, 10·9%). Light absorption: Max. at 2540 Å (ε = 10,700). The alcohol gives a yellow colour with tetranitromethane in chloroform. Acetylation of the alcohol with pyridine and acetic anhydride gave 3β-acetoxyergosta-8 : 22-dien-7-one which separated from methanol as plates, m. p. 208—210°, $[\alpha]_D$ −55° (*c*, 1·1) (Found: C, 79·0; H, 10·3. Calc. for $C_{30}H_{46}O_3$: C, 79·2; H, 10·2%). Light absorption: Max. at 2540 Å (ε = 10,000).

3β-*Acetoxy*-9β : 11α-*dihydroxyergost*-22-*en*-7-*one* (XVI; R′ = H).—(*a*) Ergosteryl-D acetate epoxide (1·0 g.) in dry chloroform (10 c.c.) was treated dropwise during 15 minutes at 0° with a solution of bromine (1 mol.) in chloroform (5 c.c.) with stirring. A solution of perbenzoic acid (1·5 mols.) in chloroform (20 c.c.) was then added during 1 hour at −5° and the mixture kept for 2 days at 0°. The mixture was evaporated to dryness under reduced pressure at room temperature, and the residue dissolved in glacial acetic acid (25 c.c.) and treated with zinc dust (10 g.) added in portions during 5 hours with stirring on the steam bath. The debrominated product was isolated by means of ether; it formed a crystalline solid which was recrystallised from acetone, to give 3β-acetoxyergosta-8 : 22-dien-7-one (250 mg.) as plates, m. p. 198—205° undepressed on admixture with an authentic specimen. The acetone mother-liquors were evaporated and a solution of the residue (0·80 g.) in ethanol (20 c.c.) treated with glacial acetic acid (0·5 c.c.) and Girard's reagent T (0·5 g.). The mixture was refluxed for 90 minutes, cooled, and diluted with water (20 c.c.) containing crushed ice, and the pH was adjusted to 5·5—6 by sodium carbonate solution. The mixture was extracted with ether (2 × 20 c.c.) (extract A). The pH of the aqueous layer was adjusted to 2—3 by hydrochloric acid, the mixture extracted with ether (2 × 20 c.c.), and the extract washed with 5% sodium carbonate solution and then water, and dried (Na_2SO_4). Removal of the ether gave a solid (80 mg.) which after three crystallisations from methanol gave 3β-*acetoxy*-9β : 11α-*dihydroxyergost*-22-*en*-7-*one* as rectangular plates, m. p. 260—262°, $[\alpha]_D$ −66° (*c*, 1·3) (Found: C, 74·0; H, 10·0. $C_{30}H_{48}O_5$ requires C, 73·7; H, 9·9%). The compound gives a pale yellow colour with tetranitromethane and does not show high-intensity absorption above 2200 Å. Evaporation of extract A followed by crystallisation from methanol gave 3β-acetoxyergosta-8 : 22-dien-7-one (200 mg.).

(*b*) Oxidation of ergosteryl-D acetate epoxide (1 g.) was effected as described above, with the difference that the reaction time with perbenzoic acid was 7 days. The debrominated product was crystallised once from acetone, to give plates, m. p. 190—210°. This solid (160 mg.) in benzene (25 c.c.) was chromatographed on a column of Grade II alumina (12 × 1·5 cm.), and the column washed with benzene (500 c.c.) which gave 3β-acetoxyergosta-8 : 22-dien-7-one (26 mg.) as plates, m. p. 203—207°, from methanol. Light absorption: Max. at 2540 Å (ε = 9100). Further elution of the column with benzene containing 1% of methanol (160 c.c.) gave a solid (105 mg.) which after three crystallisations from methanol gave 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one as rectangular plates, m. p. 261—263° (undepressed by the specimen described above), $[\alpha]_D$ −69° (*c*, 1·1) (Found: C, 73·5; H, 9·95%). The compound does not show high-intensity absorption above 2200 Å and gives a light yellow colour with tetranitromethane in chloroform.

The acetone mother-liquor was evaporated and chromatographed as above, to give 3β-acetoxyergosta-8 : 22-dien-7-one (60 mg.) together with 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one which separated from methanol as plates (40 mg.), m. p. 257—260° alone or mixed with the specimen described above; it does not show selective absorption of high intensity in the ultraviolet region of the spectrum.

(*c*) Ergosteryl-D acetate epoxide (1 g.) was oxidised with perbenzoic acid as described in (*a*) and the solution kept at 0° for 3 weeks. The reaction mixture was debrominated by zinc and acetic acid, and the product directly crystallised from acetone, to give 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one (130 mg.) as plates, m. p. 257° undepressed on admixture with the specimen described above. Chromatography of the mother-liquors gave a further 45 mg. of this compound.

3β : 11α-*Diacetoxy*-9β-*hydroxyergost*-22-*en*-7-*one* (XVI; R′ = Ac).—A solution of 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one (130 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was kept at room temperature overnight. Isolation of the product by means of ether, followed by two crystallisations from light petroleum (b. p. 60—80°) and two from methanol, gave the

diacetate as needles, m. p. 197—198°, $[\alpha]_D$ −44° (*c*, 1·0) (Found: C, 72·1; H, 9·6. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5%). This gives a pale yellow colour with tetranitromethane in chloroform and does not show high-intensity absorption above 2200 Å.

3β : 11α-*Diacetoxyergosta*-8 : 22-*dien*-7-*one* (XV; R′ = Ac).—3β : 11α-Diacetoxy-9β-hydroxyergost-22-en-7-one (100 mg.) was heated under reflux with aqueous methanolic potassium hydroxide (7 c.c.; 5%) for 8 hours. The solution was concentrated, and the reaction product isolated by means of ether. A solution of this solid in pyridine (1 c.c.) and acetic anhydride (2 c.c.) was heated on the steam-bath for 2 hours. Isolation by means of ether gave a solid (60 mg.) which after crystallisation from methanol gave 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one as hard, flat needles, $[\alpha]_D$ +14° (*c*, 0·6), m. p. 175—177° alone or mixed with the preparation described above (Found: C, 75·0; H, 9·55. Calc. for $C_{32}H_{48}O_5$: C, 75·0; H, 9·4%). Light absorption: Max. at 2520 Å (ε = 10,400). The compound gives a faint yellow colour with tetranitromethane in chloroform. 3β : 11α-Diacetoxyergosta-8 : 22-dien-7-one was also obtained as flat needles, $[\alpha]_D$ +14° (*c*, 0·6), m. p. 174—176° undepressed by the specimen described above, by treatment of 3β-acetoxy-9β : 11α-dihydroxyergost-22-en-7-one with alkali followed by acetylation of the product.

7 : 11-*Diketoergost*-22-*en*-3β-*yl Acetate* (XIII).—(*a*) To a solution of 3β-acetoxy-9α : 11α-epoxyergost-22-en-7-one (0·60 g.; m. p. 208—213°) in ethanol (65 c.c.) was added potassium hydroxide (10·0 g.) in water (15 c.c.), and the mixture was heated under reflux for 16 hours. The product was isolated by means of ether, dried by evaporation of its solution in benzene, and acetylated on the steam bath with pyridine (10 c.c.) and acetic anhydride (10 c.c.) for 1 hour. Isolation of the product by ether gave an orange-brown gum, a solution of which in benzene (40 c.c.) was adsorbed on a column (10 × 2 cm.) of Grade II alumina; the column was washed with benzene. Evaporation of the benzene filtrate (80 c.c.) gave a solid (180 mg.) which crystallised from methanol. Two recrystallisations from the same solvent gave the *compound*, $C_{32}H_{52}O_5$, as fine needles, m. p. 176—177°, $[\alpha]_D$ −15° (*c*, 0·7) (Found: C, 74·4; H, 10·0; OEt, 9·5. $C_{32}H_{52}O_5$ requires C, 74·4; H, 10·1; OEt, 8·7%). The compound gives a faint yellow colour with tetranitromethane in chloroform and does not show high-intensity light absorption above 2200 Å. Evaporation of the second benzene filtrate (50 c.c.) gave a pale yellow solid (120 mg.), crystallisation of which from methanol gave a solid (40 mg.). Crystallisation from methanol gave the compound $C_{32}H_{52}O_5$ as needles, m. p. 174—176° undepressed when mixed with the specimen described above. Evaporation of a third benzene filtrate (110 c.c.) gave a gummy solid (15 mg.) which was not further examined. The fourth benzene fraction (280 c.c.) gave a solid (45 mg.) which on crystallisation from methanol gave 7 : 11-diketoergost-22-en-3β-yl acetate as clusters of needles, m. p. 196—198°, $[\alpha]_D$ −28° (*c*, 0·9) (Found: C, 76·5; H, 10·1. Calc. for $C_{30}H_{46}O_4$: C, 76·55; H, 9·85%). The diketone does not show high-intensity light absorption above 2200 Å. A mixture with a specimen {m. p. 197—199°, $[\alpha]_D$ −31°; prepared as described by Heusser *et al.* (*loc. cit.*) who record m. p. 195—196°, $[\alpha]_D$ −27°} had m. p. 196—199°. Chamberlin *et al.* (*loc. cit.*) give m. p. 197—200°, $[\alpha]_D$ −30°, for this acetate.

(*b*) (With R. C. Anderson.] A solution of 3β : 11α-diacetoxyergosta-8 : 22-dien-7-one (140 mg.) in ethanol 66 c.c.) was treated with 50% aqueous potassium hydroxide (24 c.c.), and the mixture refluxed for 14 hours. The product isolated by means of ether and dried by evaporation of its solution in benzene was acetylated with pyridine and acetic anhydride. The acetylated product was isolated by means of ether and its solution in benzene (25 c.c.) was chromatographed on Grade II—III alumina (8 × 1·25 cm.), and the column washed with benzene. Evaporation of the first fraction (270 c.c.) gave a crystalline solid (20 mg.), m. p. 155—170°. The next fraction (200 c.c.) yielded a solid (15 mg.) which after two crystallisations from methanol gave 7 : 11-diketoergost-22-en-3β-yl acetate as small prismatic needles, m. p. 196—198°, $[\alpha]_D$ −25° (*c*, 0·5) (Found: C, 76·4; H, 9·9. Calc. for $C_{30}H_{46}O_4$: C, 76·5; H, 9·85%). The compound did not show high-intensity absorption above 2200 Å. It was undepressed in m. p. when mixed with an authentic specimen.

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